CLS 281

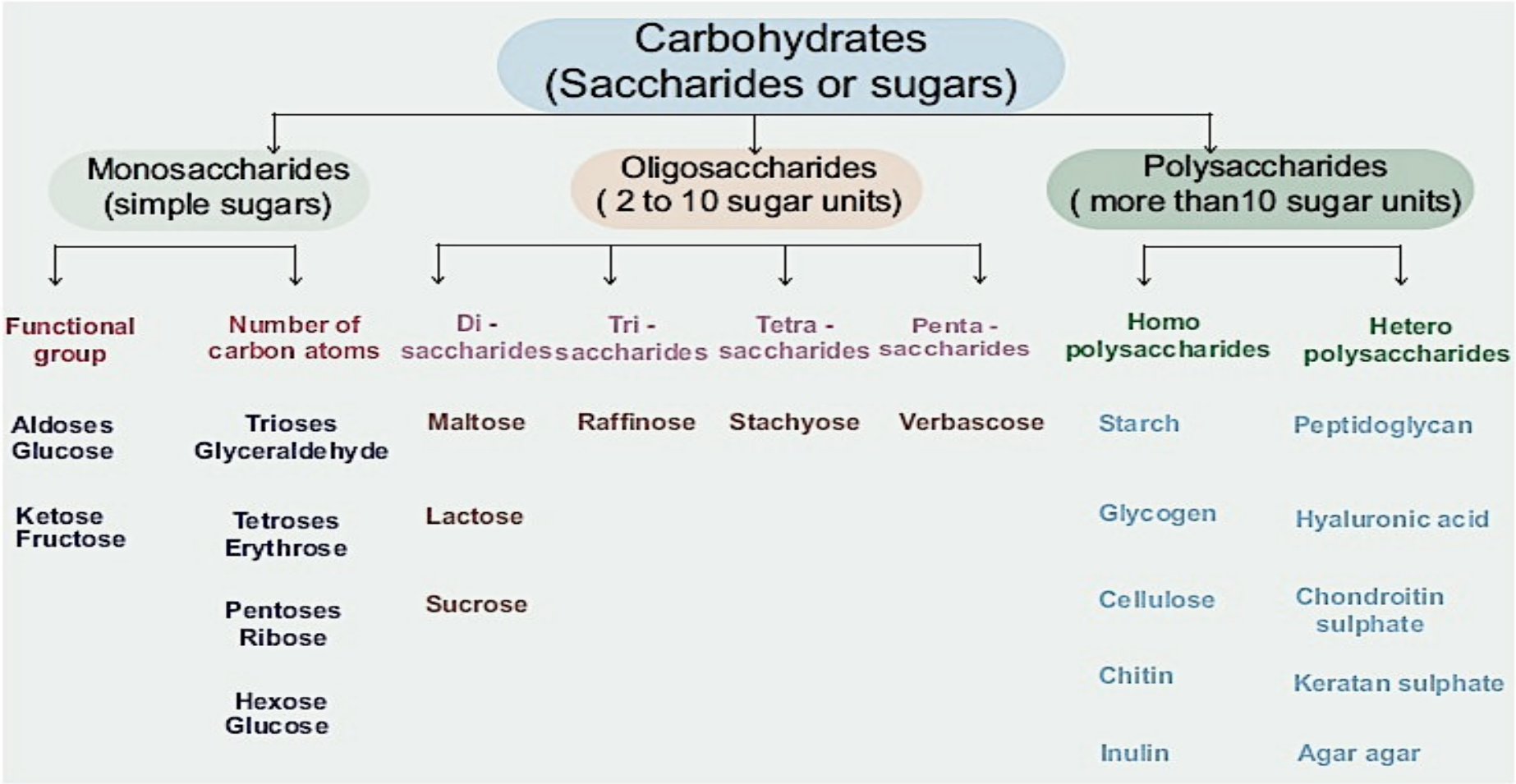
Basic Biochemistry and Biomolecules

جامعة الملك سعود
King Saud University
1957

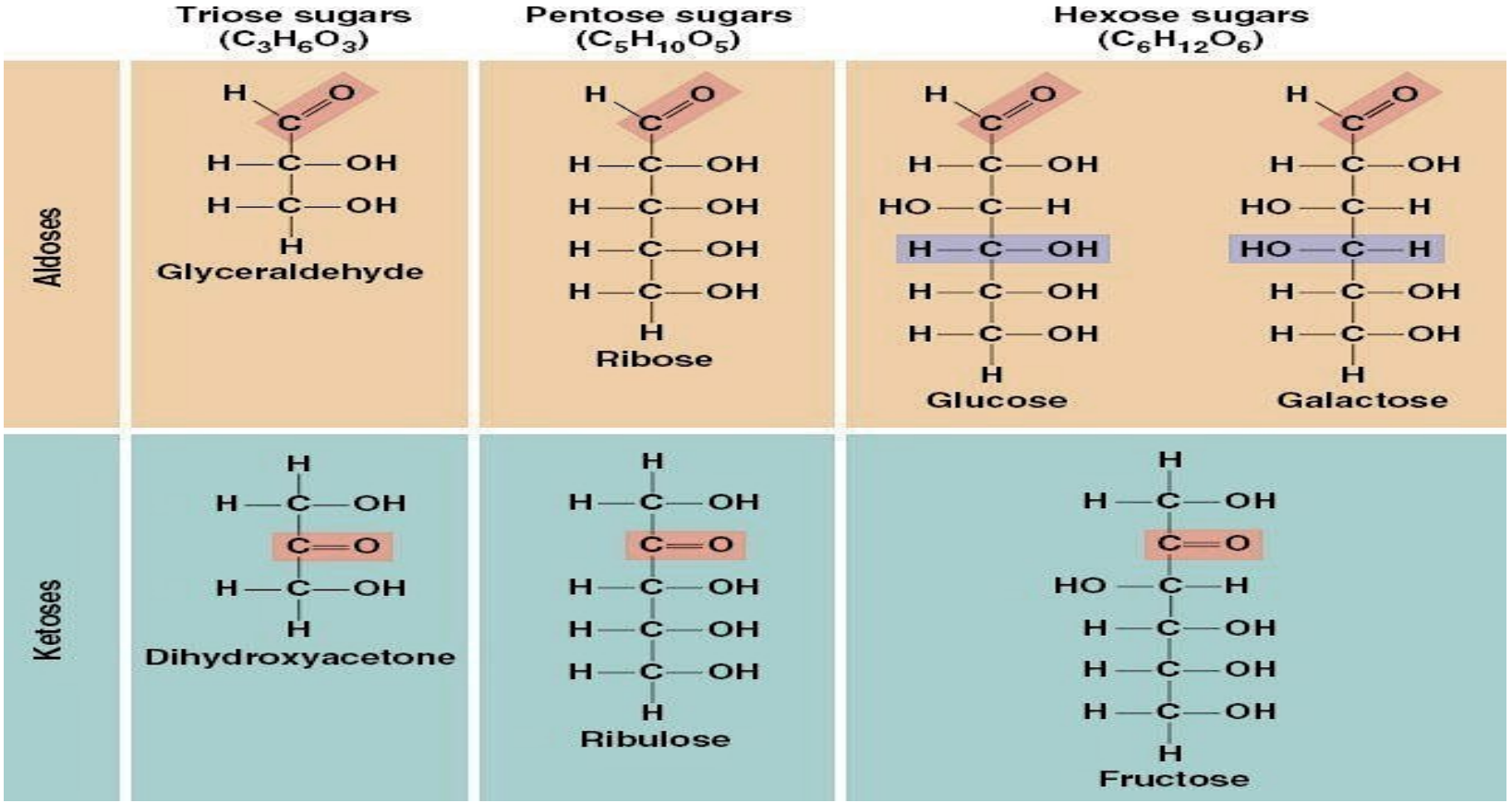
Experiment 4

General Color Tests for Carbohydrates

Carbohydrate Classification



Aldose vs Ketose

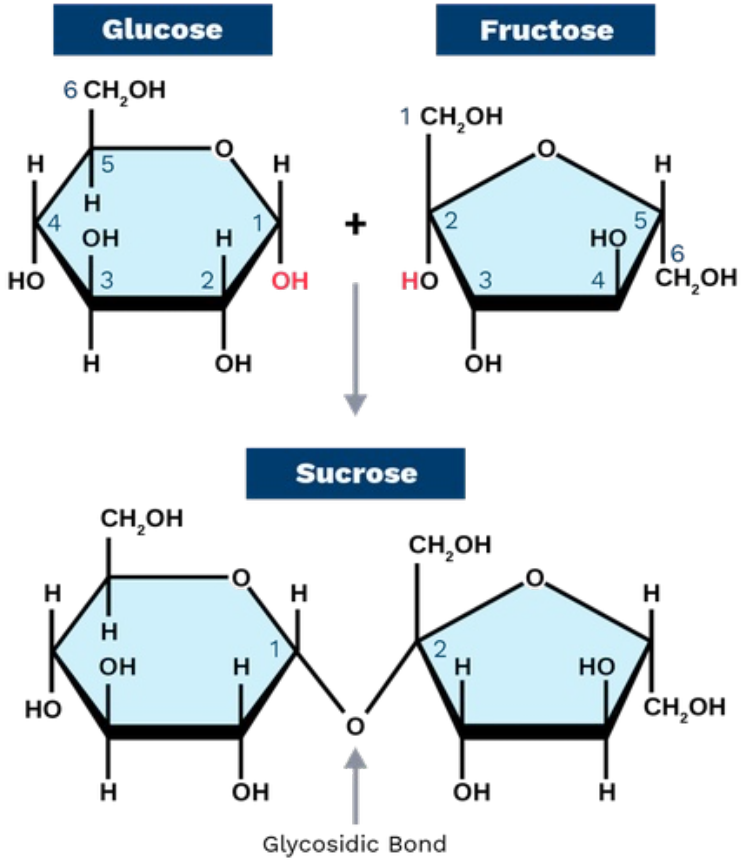


Aldose has a carbonyl group at the end of the carbon chain.
Ketose has a carbonyl group in the middle of the carbon chain.

Two monosaccharides are joined together by a

Glycosidic Bond

formed by the loss of water molecule.



General Color Tests for Carbohydrates

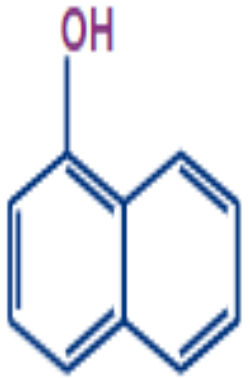
1. Molisch Test
2. Anthrone Test

01 Molisch Test

- It is a general test used to detect the presence of carbohydrates in a given sample.

- **Molisch's Reagent**

  - concentrated sulphuric acid H_2SO_4
  - Phenol-type molecules (such as α-naphthol or thymol).
    - α-Naphthol.
    - Thymol:
      - It can be used as a reagent instead of α-Naphthol.
      - Thymol is more stable than α-Naphthol and can be applied to insoluble carbohydrates like cellulose or wood.



α-naphthol

01 Molisch Test Principle

- **Principle**

  Heat

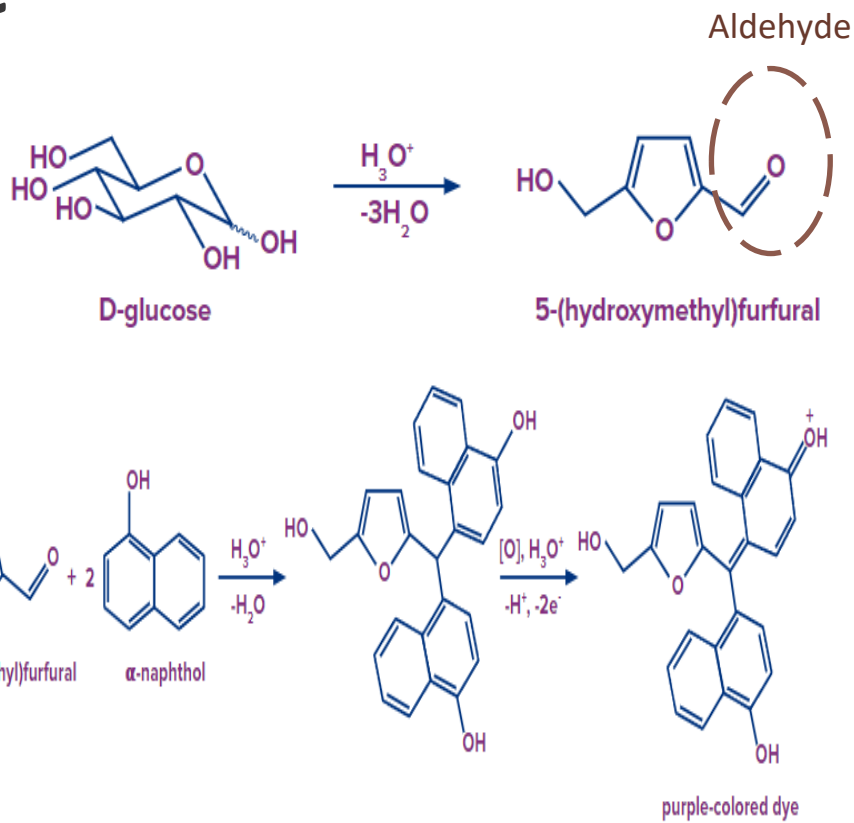
  Monosaccharides + H2SO4 → furfrual (of furfural derivatives) + 3 H2O

  furfrual (of furfural derivatives) + α-Naphthol (2 phenol groups) → **purple ring** at the interface.

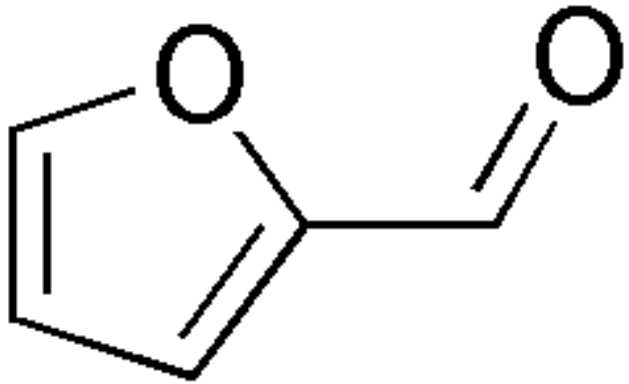
- Carbohydrate undergoes dehydration upon the introduction of concentrated sulphuric acid, resulting in the formation of an aldehyde.

- This aldehyde undergoes condensation along with two α-naphthol, resulting in the formation of a purple or reddish-purple-colored complex.

- The **purple ring color** is due to condensation products of furfural or its derivatives with α-Naphthol.



Furfural



- Furfural is an organic compound derived from a variety of agricultural byproducts, including corncobs, oat, and wheat bran.
- The name furfural comes from the Latin word furfur, meaning bran, referring to its usual source.
- It is not a carbohydrate.

01 Molisch Test

- It is effective for any compound which can be dehydrated to furfural or substituted furfural (such as hydroxymethyl furfural) by concentrated sulfuric acid.

- If the carbohydrate is an oligosaccharide (e.g., disaccharide, trisaccharide ...etc.) or a polysaccharide, the hydrolysis of the carbohydrate acetal linkage occurs simultaneously with the dehydration reaction (in polysaccharide, the color develops slower).

01 Molisch Test Procedure

Note: swirl the samples and reagent bottles before use to aspirate homogonous solution.

Steps	Tube No.	Tube 1	Tube 2	Tube 3
1	Sample	2 ml of 0.5% starch	2 ml of 0.5% sucrose	2 ml of 0.5% glucose
2	α-Naphthol Reagent	2 drops	2 drops	2 drops
3	Mix			
4	Con. H_2SO_4 Reagent	2 ml	2 ml	2 ml

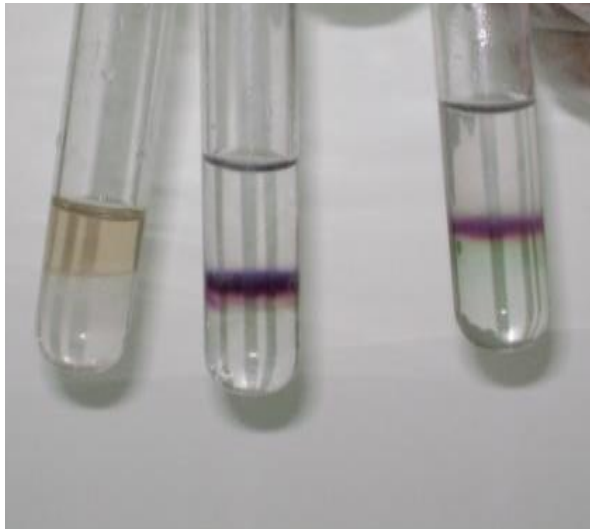
Positive result >>> purple ring at the interface indicate the presence of carbohydrate.

- Incline the test tube slowly and carefully add 2 ml of concentrated sulfuric acid (H_2SO_4) down the side of the tube to form a layer below the sugar solution.

01 Molisch Test Result

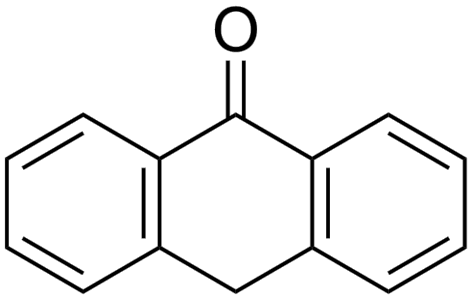
- All carbohydrates are detected using this test.
- Monosaccharides give a rapid positive test.
- Disaccharides and polysaccharides react slower.

- A negative result by this reaction is very good evidence of the absence of carbohydrates, but a positive test is an indication of the probable presence of carbohydrates.



02 Anthrone Test

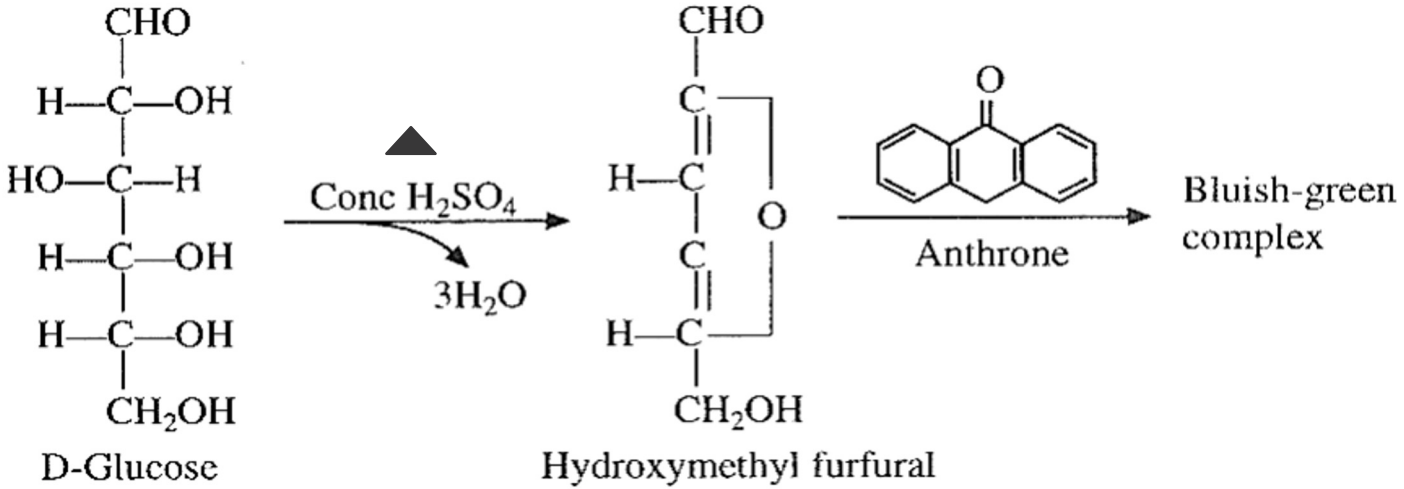
- It is another general test for carbohydrates.
- **Aim:** To detect the presence of carbohydrates in a given solution.
- **Reagent**
  - Concentrated sulphuric acid H_2SO_4
  - Phenol-type molecules (anthrone)



Anthrone

- Based on similar reactions in which anthrone is used instead of α-Naphthol in the Molisch test to form a green to blue-green color product.

02 Anthrone Test Principle



1. Hydrolysis of sugar to monosaccharides.
2. Dehydration of monosaccharides by H2SO4 producing furfural (of furfural derivatives).
3. The reaction of furfural with Anthrone reagent resulted in **a Blue-green complex.**

02 Anthrone Test Procedure

Steps	Tube No.	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5
1	Sample	Small piece of Filter paper	1 drop 0.5%starch	1 drop of 0.5%sucrose	1 drop 0.5%glucose	1 drop Blank
2	Water As a Diluent	1ml	1ml	1ml	1ml	1ml
3	Anthrone reagent	3ml	3ml	3ml	3ml	3ml

4. Heat in boiling water bath for 3 mins.

02 Anthrone Test

- It is very sensitive.
- It will give a positive reaction with filter paper (cellulose).

Uses:

- It can be used for the quantitative determination of glycogen and sugar in the blood.
- It can be used as a qualitative test since different sugars dehydrate at different rates and produce a variety of colors.

Summary of General Color Tests for Carbohydrates

Test	Detect	Reagent	Principle	Positive Result	Negative Result	Note
Molisch Test	Detect the presence of **carbohydrates** in a given sample.	- concentrated sulphuric acid H_2SO_4. - Phenol-type molecules **(α-Naphthol).**	1- Hydrolysis of sugar to monosaccharides. 2-Monosaccharides + H_2SO_4 → furfrual (of furfural derivatives) + 3 H_2O (dehydration reaction) 3- furfrual (of furfural derivatives) + α-Naphthol (2 phenol groups) → **purple ring** at the interface. (condensation reaction)	**purple ring color**	Colorless	Monosaccharides give a **rapid** positive test. Disaccharides and polysaccharides react **slower**.
Anthrone Test	Detect the presence of **carbohydrates** in a given sample.	- concentrated sulphuric acid H_2SO_4. - Phenol-type molecules **(anthrone)**	1- Hydrolysis of sugar to monosaccharides. 2-Monosaccharides + H_2SO_4 → furfrual (of furfural derivatives) + 3 H_2O (dehydration reaction) 3-The reaction of furfural with Anthrone reagent resulted in **a Blue-green complex.**	**Blue-green complex.**	Reagent color	It is very sensitive. It will give a positive reaction with filter paper (cellulose).

Guideline for writing the lab report

Total: 5 marks

All the following information should be included in your report:

a) Course # (CLS 281)
b) Experiment title
c) Date of the experiment
d) Student's names and university ID#
e) Section #

The lab report is broken down into 6 sections:

1. Experiment **title**
2. The **aim** of the experiment (objective, or what the test detects specifically) (1 mark)
3. **Principle** (chemical reaction) (1 mark)
4. **Methodology** (written in **steps**, not in tables)
5. **Result** (1 mark)
6. **Interpretation or Comme**nt (2 mark)

Deadline: Next lab Submission: via email